FACULDADE DE MEDICINA DA UNIVERSIDADE DE COIMBRA

TRABALHO FINAL DO 6º ANO MÉDICO COM VISTA À ATRIBUIÇÃO DO GRAU DE MESTRE NO ÂMBITO DO CICLO DE ESTUDOS DE MESTRADO INTEGRADO EM MEDICINA

SARA MANSO VENTURA

CLINICAL BEHAVIOR OF SMALL CELL LUNG CANCER

ARTIGO CIENTÍFICO

ÁREA CIENTÍFICA DE PNEUMOLOGIA

TRABALHO REALIZADO SOB A ORIENTAÇÃO DE:
PROFESSORA DOUTORA LINA CARVALHO
DRA. ALICE MARIANO PÊGO

[11/2009]

Abstract

Introduction: Lung Cancer is the leading cause of cancer death in the United States and Western Europe. Small cell lung cancer (SCLC) accounts for up to 13% of all newly diagnosed lung cancers. The knowledge of factors that could predict the clinical outcome of patients with SCLC is important for guiding treatment and to determining prognosis.
Imunohistochemical study by the analysis and characterization of markers involved in SCLC could improve the knowledge of prognostic factors. The purpose of this article is to investigate the prognostic value of six Imunohistochemical Markers in patients with SCLC diagnosis: Chromogranin A (Chromo A), Cytokeratin 7 (CK7), Thyroid transcription factor-1 (TTF-1), Neural cell adhesion molecule/CD56, Ki-67 (MIB1) and High weight cytokeratin (LP34).

Material and Methods: Patients had a histological confirmed small-cell lung cancer (SCLC) diagnosed at our University Hospital between February 2002 and December of 2008 and a total of 100 cases (13 women, 87 men) were selected for this study.

Results: The mean survival of all patients was 274 days (9months), and median was 183 days (6months). The survival mean for LD-SCLC was 482 days (16months, with a 95% Confidence Interval from 10 months to 22 months) and 182 days for ED-SCLC (6months, with a 95% Confidence Interval from 4 months to 8 months).
The patients showed a significant meaning in Chromo A curves isolated, with a mean of Survival with the group expressing A of 6,7 months and 11 months in the group

expressing B. Tumors that expressed Chromo A (B) & CD56 (A) had the best prognosis with a survival mean of 726 days (24,2months) against 234 days (7,8 months). The worst prognosis was for the combination of CD56 (B) + Ki67 (B) with a survival mean of 6 months against 11 months for the rest of the patients.

Conclusion: Based on those considerations, we hypothesize that Chromo A isolated could be a prognostic factor of survival. Patients that show Chromo A (B) & CD56 (A) had a very good prognosis with a mean of survival of 726 days. On other hand, patients that show CD56 (B) + Ki67 (B) had a poor prognosis with a mean of survival of 6 months.

Keywords: small cell lung cancer, prognostic factors, Imunohistochemical Markers, Ki67, LP34, CK7, Chromo A, CD56, TTF1.

Introduction

Lung Cancer is the malignant leading cause of death in the United States and Western Europe by Cancer. Small cell lung cancer (SCLC) accounts for up to 13% of all newly diagnosed lung cancers and is strongly associated with cigarette smoking. It differs from other types of lung cancer in its propensity for early systemic spread and its aggressive clinical course if left untreated. (1, 2)

Together with melanoma it is considered, one of the most aggressive tumors accounting with a survival mean ranging from 2 to 4 months without treatment. With currently available chemotherapy, the survival mean for patients with LD-SCLC (limited stage disease SCLC), ranges from 16 months to 26 months. Patients with tumors that have metastasized to contralateral supraclavicular nodes, cytology-proven malignant pleural or pericardial effusion are staged as extensive-stage disease SCLC (ED-SCLC), and the mean survivals varies from 6 months to 12 months. (3-6)

It was demonstrated previously that good performance status (PS), young age, being a woman and limited stage disease are associated with an improved prognosis. Other variables like liver metastasis, low albumin levels, and low sodium levels have been associated with a poor prognosis. (7-13)

Compared with other cell types of lung cancer SCLC has different biological behaviors. The knowledge of factors that could predict the clinical outcome of patients with SCLC is important for guiding treatment and to determining prognosis.

Imunohistochemical study by the analysis and characterization of markers involved in SCLC could improve the knowledge of prognostic factors. The six imunohistochemical

markers used in this trial were: Chromogranin A (CromoA), Cytokeratin 7 (CK7), Thyroid transcription factor-1 (TTF-1), Neural cell adhesion molecule/CD56, Ki-67 (MIB1) and High weight cytokeratin (LP34).

Chromogranin A (Chromo A) is an acidic glycoprotein belonging to a family of regulated secretory proteins stored in the dense core granules of the adrenal medulla and of many other neuroendocrine cells and neurons. The role of Chromo A is not known precisely, but possible functions include intracellular regulation of the formation of neuroendocrine granules, regulation of hormone secretion and function as a pro-hormone. (14)

Cytokeratins (CK) are soft epithelial intermediate filaments that comprise approximately 20 different Keratin polypeptides. This family of intermediate filaments is found to be crucial in the diagnostic immunohistochemistry and for identification of specific carcinoma subtypes. Cytokeratin 7 (CK7) is a simple keratin that has restricted distribution in many simple, stratified and ductal epithelium, such as breast, ovary, lung, uterus and neuroendocrine cells. (15)

Monoclonal antibody 34betaE12 (or LP34) recognizes a set of cytokeratins (1, 5, 10, 14) expressed in normal stratified squamous epithelium. Some trials have recently reported its expression in squamous cell carcinoma and basaloid carcinoma, in contrast to large cell neuroendocrine carcinoma, an entity with overlapping morphological features with basaloid carcinoma.

Thyroid transcription factor-1 (TTF-1) is a tissue specific homeodomain containing DNA-binding proteins of the Nkx-2 gene family. It plays an important role in the early differentiation and morphogenesis of the developing brain, lung and thyroid. In lungs, it activates the promoters for Clara cell secretory protein, and surfactant apoproteins.

Different percentages in TTF-1 nuclear expression are of clinical importance to distinguishing different histological types of lung carcinoma and it is still incompletely understood its high expression in SCLC. (16)

Neural cell adhesion molecule/CD56 is specifically expressed by neural, peripheral neuroectodermal and neuroendocrine tissues and tumors. It belongs to the immunogloblulin family of cell surface adhesion proteins involved in direct cell-cell adhesion. CD56 is also found in natural killer cells, natural killer-like T cells, myocites, and seromucous glands. (17)

Ki-67 antigen in tumor cell reflects the expression of a DNA-binding nuclear protein encoded by a gene in chromosome 10. Moreover, it is known that Ki6 expression is a molecular marker of tumor proliferation, and its over-expression has been linked to a poorer prognosis in NSCLC. (18)

The purpose of this work was to investigate the prognostic value of the described Imunohistochemical Markers, usually applied in daily routine of SCLC diagnosis.

Material and methods

Human Subject

Patients had a histological confirmed small-cell lung cancer (SCLC) diagnosed at our University Hospital between February 2002 and December of 2008 and a total of 100 cases (13 women, 87 men) were selected for this study.

All patients who were ineligible for absence of survival register and were alive with a survival period bellow one year were excluded. Histological diagnosis was made according to the World Health Organization guidelines. The following clinical variables were registered: age at diagnosis time, symptoms that lead to diagnosis, smoking history, extension of disease (ED-SCLC and LD-SCLC), pathological antecedents and survival.

The mean age of diagnosis was 65 years (range, 27-83 years).

The survival time was defined as the interval in days beginning with the histological diagnosis and death or last recorded follow-up. From the 100 patients 5 were alive on the 15th of September 2009 and their survivals were over 24 months.

Tissue Analysis - Immunohistochemistry

Three µm sections of TMA were placed on coated slides and were allowed to dry overnight. After deparaffinization and rehydration, antigen unmasking was performed using pronase E on LP34 and CK7 markers, using Module PT for Citrate buffer in Chromo A and Module PT for EDTA buffer in TTF1 and Ki67, for 10 minutes. Endogenous peroxidase activity was quenched using 15 minutes incubation in 3% diluated hydrogen peroxidase (H2O2). For blocking nonspecific binding of secondary

antibody, Ultra V Block (Ultra vision Kit; TP-015-HL; LabVision) was applied to the sections and then the sections were incubated at room temperature with primary antibodies against clone 123C3, DAKO – CD56; clone LP34, Novocastra – LP34; clone DV-TL 12/30, DAKO – CK7; clone DAK-A3, DAKO – Chromo A; clone 8G7G3/1, DAKO – TTF1 and clone MIB-1, Dako – Ki67 at a dilution of: 1:100 to CD56 for 30 minutes; 1:100 for 60 minutes to LP34; 1:50 for 30 minutes to CK7; 1:300 for 30 minutes for Chromo A; 1:100 for 60 minutes for TTF1 and 1:50 for 30 minutes to Ki67. After washing with phosphate –buffered saline (PBS) the slides were incubated with biotin-labeled secondary antibody for 30 minutes. Primary antibody biding was localizated in tissues using peroxidase-conjugated streptavidin (LabVision) and 3-3diaminobenzidine tetrahydrochloride (DAB) was used as the chromogen, according to manunfacturer's instructions. The slides were counterstained with hematoxylin, dehydrated and mounted. In parallel we used known positive controls and negative controls.

Each imunohistochemical anthibody was validated according with two variables, A and B, that correspond to 0-25% of cells expressing the antibody – A, and >25% of cells with positivity - B.

Statistical Analysis

The statistical analysis was performed with SPSS Version18.0. Lifetable probabilities of overall survival were performed by the Kaplan–Meier method (Kaplan and Meier, 1958), and differences in survival between subgroups of patients were compared with the log-rank test (Mantel, 1996). A P-value < 0.05 was considered significant.

Results

This study included 100 (13 women and 87 men) patients diagnosed with Small Cell Lung Cancer at Coimbra University Hospital between 2002 and 2008. The mean age of diagnosis was 65 years.

Of the 100 patients, only 80 (11 women and 69 men) had register of smoking history. Fifteen were non smokers (10 women) and the other 65 (81%) were smokers with a mean of 61,3 pack-years. (Fig.1)

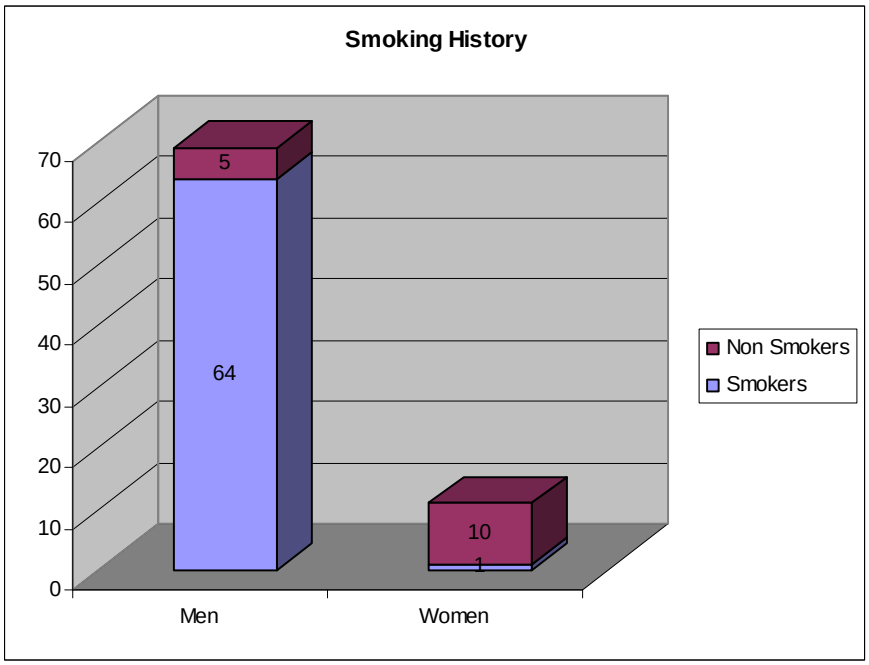

Fig. 1 – Distribution of gender by Smoking History.

The most frequent symptoms in 93 patients were cough (28,0%) dyspnea (29,8%), asthenia (23,8%) and anorexia (18,3%); incidental finding happened in 18,3%. (Table I and Fig.2).

Symptoms	n	%	Symptoms	n	%
Cough	26	28,0	**Muscle weakness (lower members)**	2	2,2
Dyspnea	24	25,8	**Painful cervical mass**	2	2,2
Asthenia	18	19,4	**Hoarseness**	2	2,2
Anorexia	17	18,3	**Pleural effusion**	2	2,2
Incidentaloma	17	18,3	**Paresthesia (left arm)**	1	1,1
Chest pain	16	17,2	**Pleuritic Chest pain**	1	1,1
Hemoptysis	13	14,0	**Aphasia**	1	1,1
Flu syndrome	12	12,9	**Wheeze**	1	1,1
Weight loss	11	11,8	**Loss of vision**	1	1,1
Osteoarticular pain	5	5,4	**Nausea**	1	1,1
Dysphagia	5	5,4	**Paresthesia (lower member)**	1	1,1
Headache	4	4,3	**Arm edema**	1	1,1
Disequilibrium	3	3,2	**Abdominal Pain**	1	1,1
Pneumonia	3	3,2	**Disorientation**	1	1,1
Dysphonia	2	2,2	**Atelectasis**	1	1,1
Orthopnea	2	2,2	**Vomitus**	1	1,1
Diabetes insipidus	2	2,2	**Anemia**	1	1,1
Vertigo	2	2,2	**Adenopatias axilares**	1	1,1
Muscle weakness	2	2,2	**Sudden paraplegia below D12**	1	1,1

Table I – Symptoms: Most of the patients have more than one symptom.

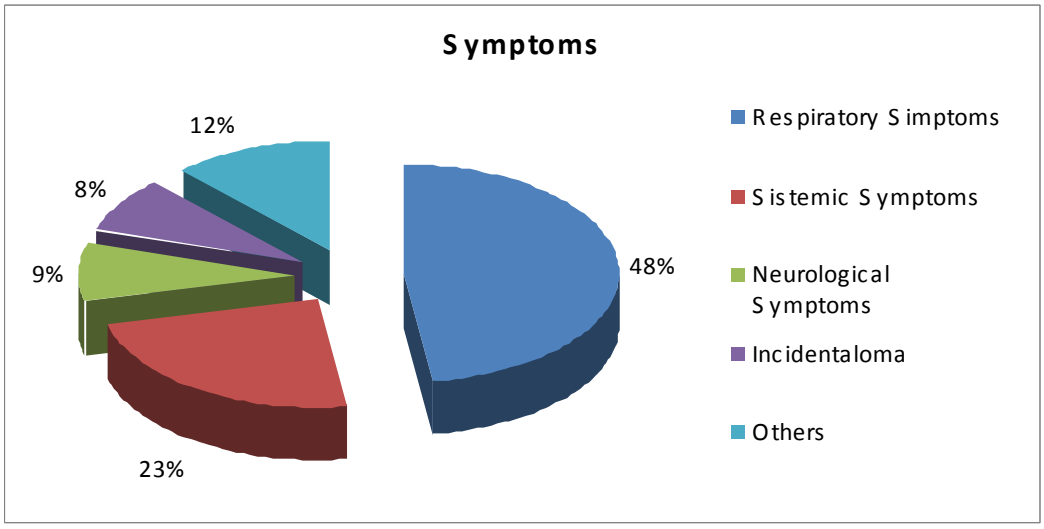

Fig.2 – Symptoms distributed by groups.

The more common pathological antecedent was HTA (19 in 79 patients) followed by absence of pathological antecedents (15 patients).

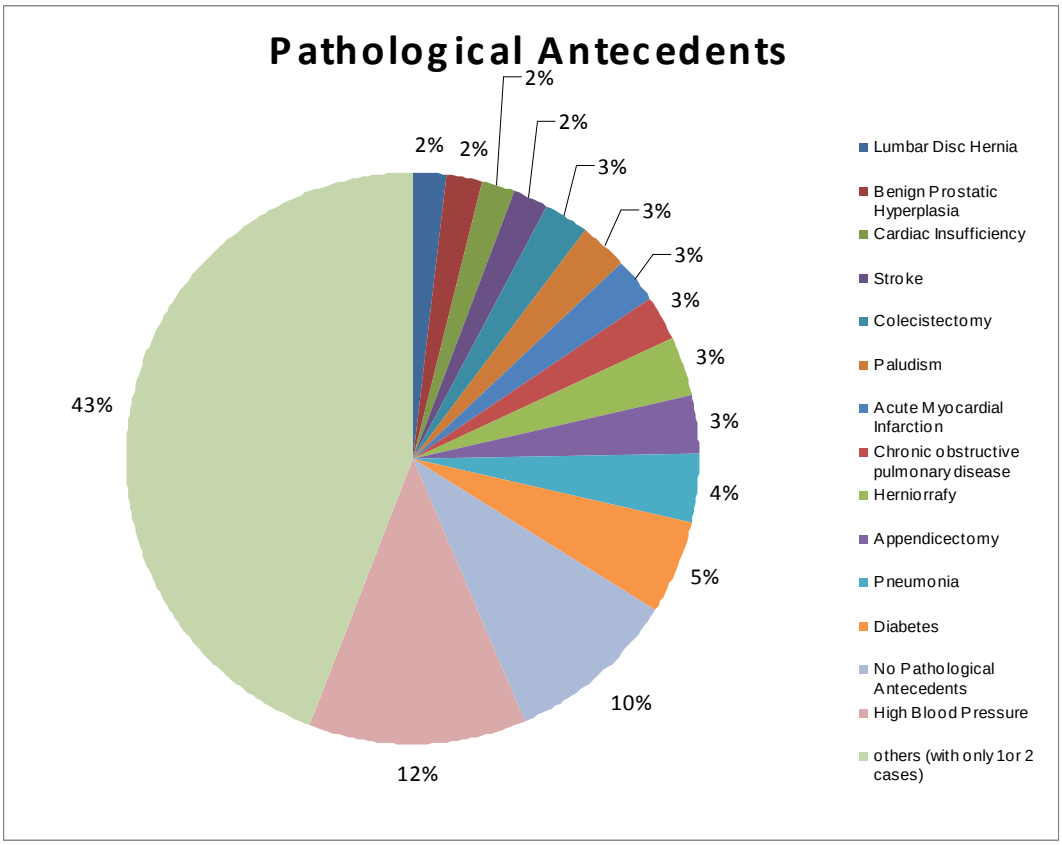

Fig. 3 – Pathological Antecedents.

The mean survival of all patients was 274 days (9months), and median was 183 days (6months); 34 patients remained alive after 274 days survival. (Table II, Fig.4, Fig. 5).

Means and Medians for Survival Time								
	Mean				Median			
			95% Confidence Interval				95% Confidence Interval	
	Estimate	Std. Error	Lower Bound	Upper Bound	Estimate	Std. Error	Lower Bound	Upper Bound
Survival	273.840	33.814	207.564	340.116	183.000	21.000	141.840	224.160

Table II – Means and Medians for Survival

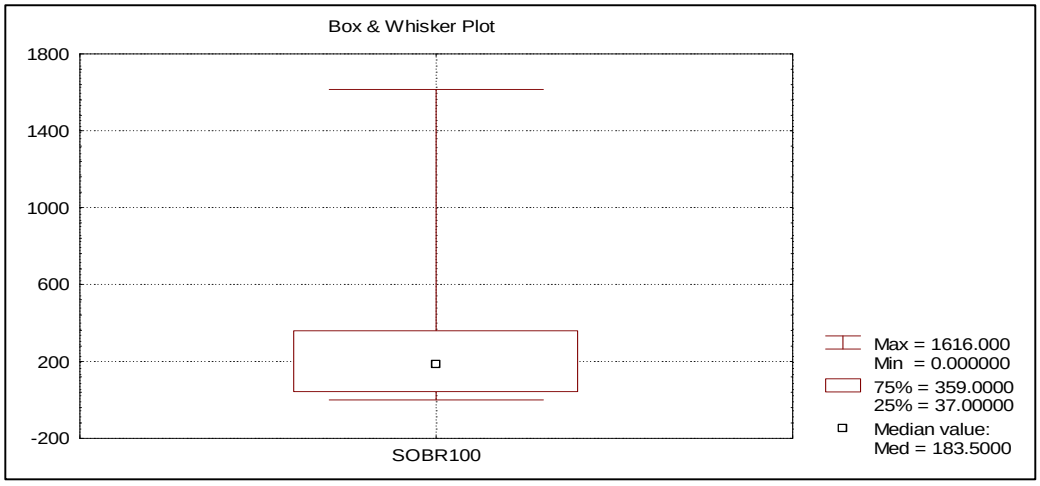

Fig.4 – Percentage of Patients Survival with SCLC

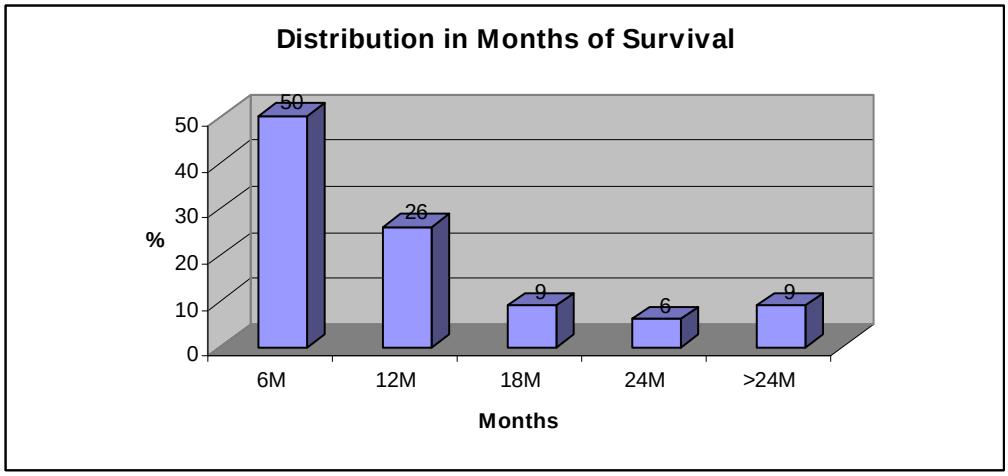

Fig.5 – Distribution of Survival Days by semesters.

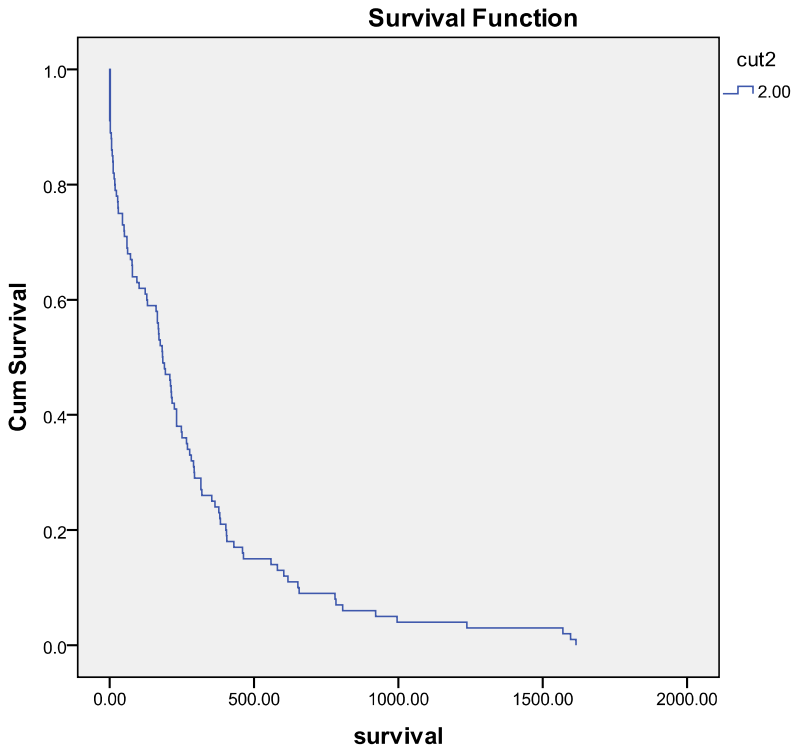

Fig. 6 – Survival of all patients. Cum Survival (Cumulative Survival – Percentage). Survival by days.

The evaluation of prognostic value of gender is defined in Fig.7 and the overall comparison of Clinical parameters is described in Table III. The relation between age and survival can be seen in Fig. 8, which represents the relation of curves between the population with age bellow 70 years and population above 70 years. That division of age was the most significant if compared with division between lower and upper 65 years, 60 years, and 75 years. The relation between extension of disease and survival is in Fig. 9, and the significance of those curves in Table III

.

Overall Comparisons			
Log Rank (Mantel-Cox)	Chi-Square	df	Sig.
Gender (Female vs Male)	.082	1	.775
Age (<70 & >70)	2.136	1	.144
Extension	13.351	1	.000

Table III - Test of equality of survival distributions for the different factors.

Sig – Significance (P value).

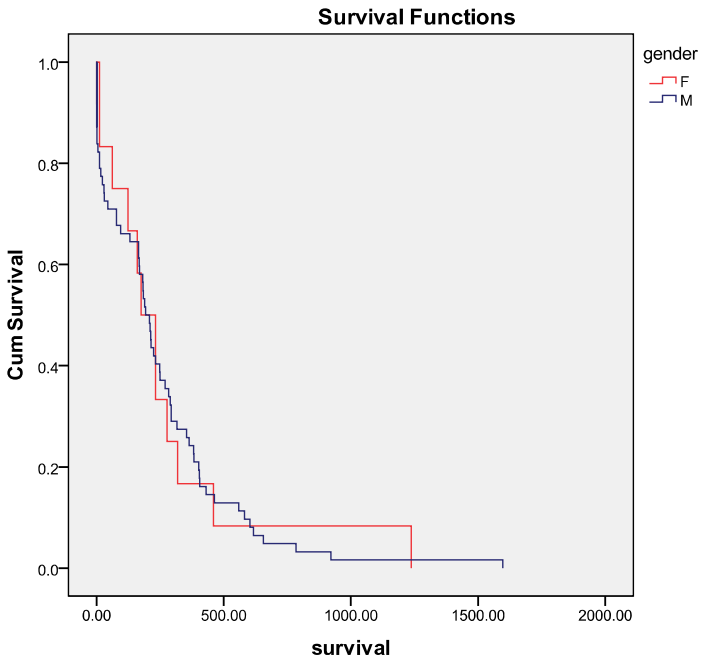

Fig. 7 – Survival Curves by Gender. Cum Survival (Cumulative Survival – Percentage). Survival by days.

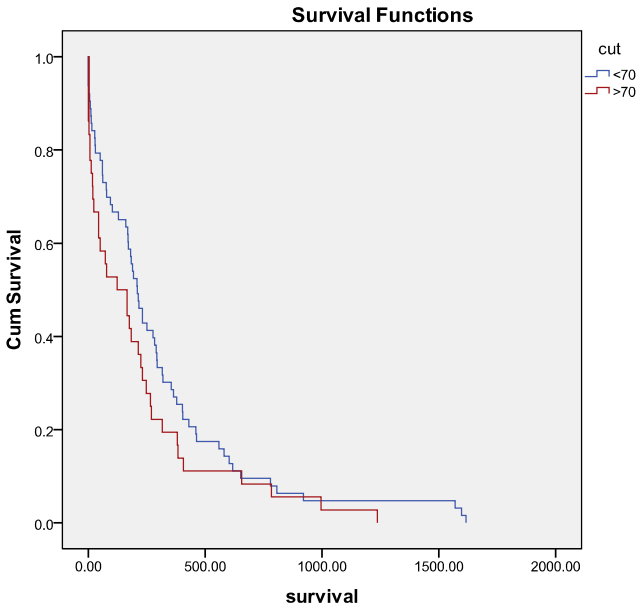

Fig. 8 – Survival Curves by Age. Upper and lower 70 years.

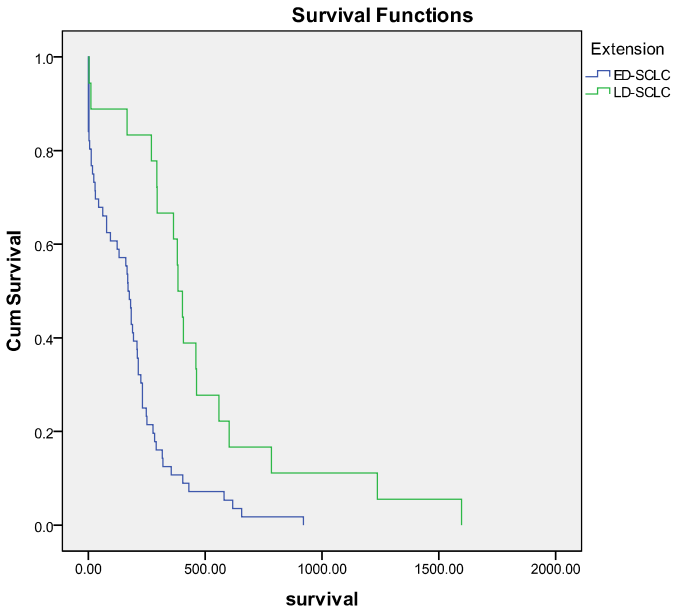

Fig. 9 – Survival Curves by Extension of SCLC. ED-SCLC (Extensive disease of Small Cell Lung Cancer) LD-SCLC (Limited Disease of Small Cell Lung Cancer).

Extensive disease was the only factor that demonstrated significant difference for survival: the survival mean for LD-SCLC was 482 days (16months, with a 95% Confidence Interval from 10 months to 22 months) and 182 days for ED-SCLC (6months, with a 95% Confidence Interval from 4 months to 8 months). The median value of survival for LD-SCLC was 383 (12.7) months and 169 (5.6months) for ED-SCLC.

Means and Medians for Survival Time								
Extension	Mean				Median			
	Estimate	Std. Error	95% Confidence Interval (days)		Estimate	Std. Error	95% Confidence Interval (days)	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
LD-SCLC	481.833	92.904	299.741	663.925	383.000	22.274	339.343	426.657
ED-SCLC	182.464	25.083	133.301	231.627	169.000	14.343	140.888	197.112
Overall	255.284	32.766	191.062	319.506	193.000	17.742	158.225	227.775

Table IV – Means and Medians for LD-SCLC and ED-SCLC.

Survival Means and range			
		95% Confidence Interval (days)	
	Mean	Lower Bound	Upper Bound
Gender (Female vs Male)			
Female	256.308	80.779	431.836
Male	276.460	204.576	348.343
Age (<70 & >70)			
<70 years	308.825	219.232	398.419
>70 years	216.583	121.866	311.301
Extension			
LD-SCLC	481.833	299.741	663.925
ED-SCLC	182.464	133.301	231.627

Table V – Survival means and Confidence Intervals (days)

The distribution of Markers Expression in all group (100 patients) is related on Table VI.

Case Processing Summary											
CK7		LP34		CromoA		TTF1		Ki67		CD56	
	N		n		n		n		n		n
A	57	A	98	A	43	A	30	A	14	A	20
B	43	B	2	B	57	B	70	B	86	B	80
Overall	100	Overall	100	Overall	100	Overall	100	Overall	100	Overall	100

Table VI - Distribution of Markers Expression. A – less then 25% positive cells expression; B – 25% to 100% positive cells expression.

The value of significance in survival distributions for the different Imunohistochemical (IMC) Markers is described in table VII. This value expresses the difference between the two curves of survival (A and B). Each curve corresponds to a variable A or B in one Imunohistochemical Marker.

Overall Comparisons			
Log Rank (Mantel-Cox)	Chi-Square	df	Sig.
CK7	.572	1	.450
LP34	.036	1	.850
CromoA	3.575	1	.059
TTF1	.585	1	.444
Ki67	.625	1	.429
CD56	1.466	1	.226

Table VII - Test of equality of survival distribution for the different antibodies.

.

Means and Medians for Survival Time

ChromoA	Mean[a]				Median			
			95% Confidence Interval				95% Confidence Interval	
	Estimate	Std. Error	Lower Bound	Upper Bound	Estimate	Std. Error	Lower Bound	Upper Bound
A	202.488	30.557	142.596	262.381	175.000	19.011	137.738	212.262
B	327.667	53.829	222.161	433.172	193.000	45.831	103.171	282.829
Overall	273.840	33.814	207.564	340.116	183.000	21.000	141.840	224.160

Table VIII - Means and Medians for population expressing A and B for Chromo A.

When we evaluate all variables (A and B) in each IMQ Mark we only found statistically meaning for Chromo A. The Fig. 10, 11, 12, 13, 14 and 15 shows the survival distribution of variables in each Mark.

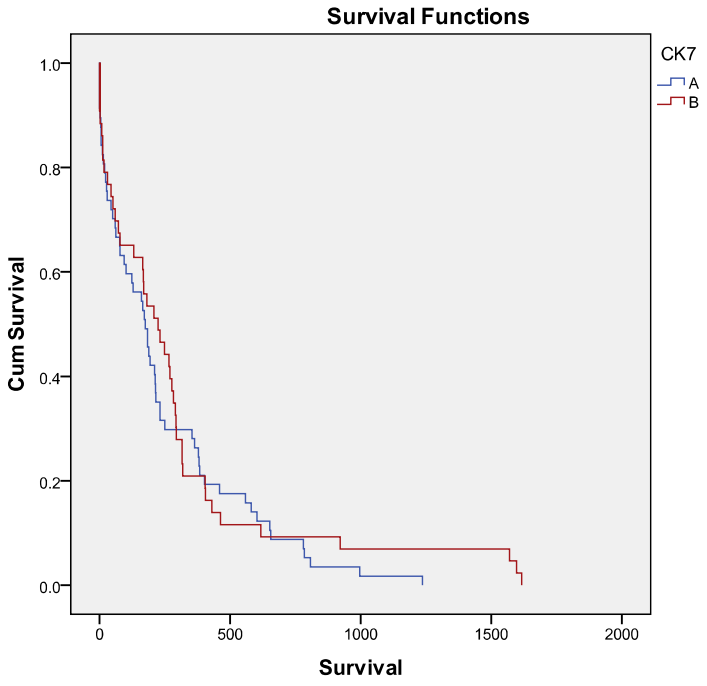

Fig. 10 – Survival Curves in CK7

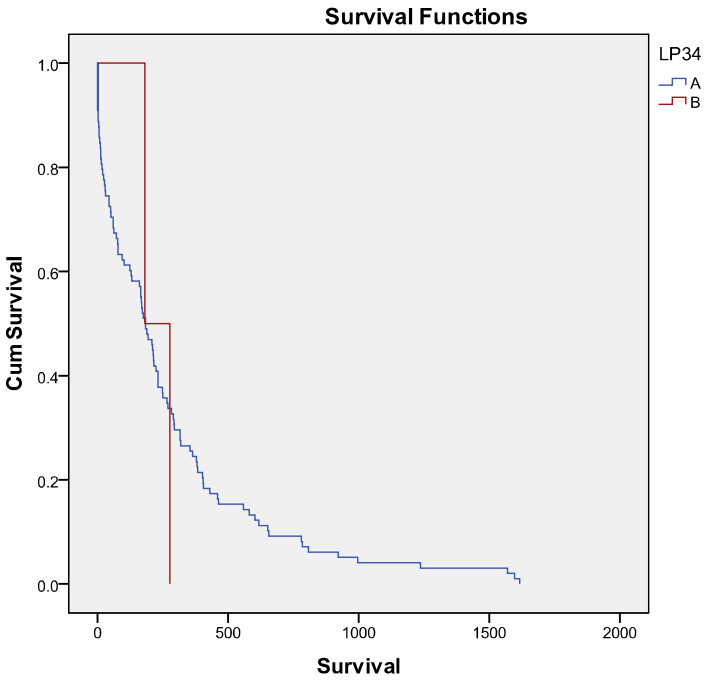

Fig. 11 – Survival Curves in LP34.

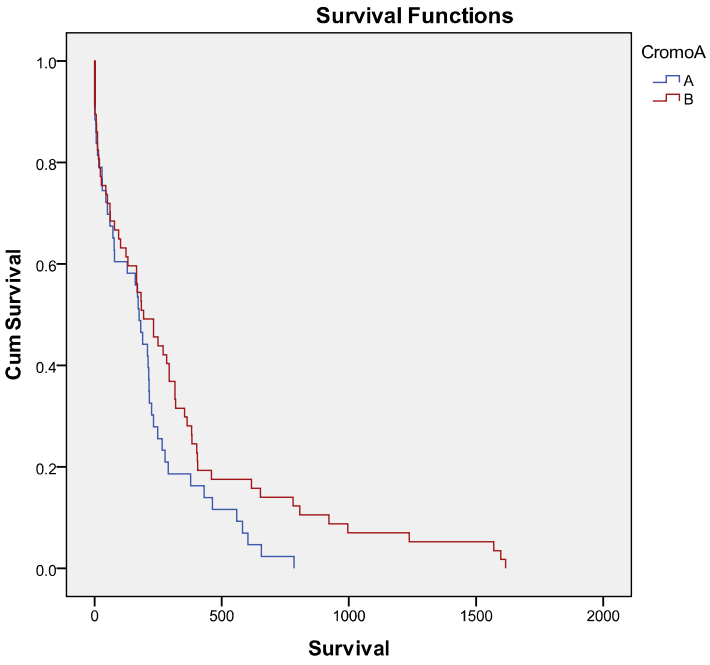

Fig. 12 – Survival Curves in CromoA.

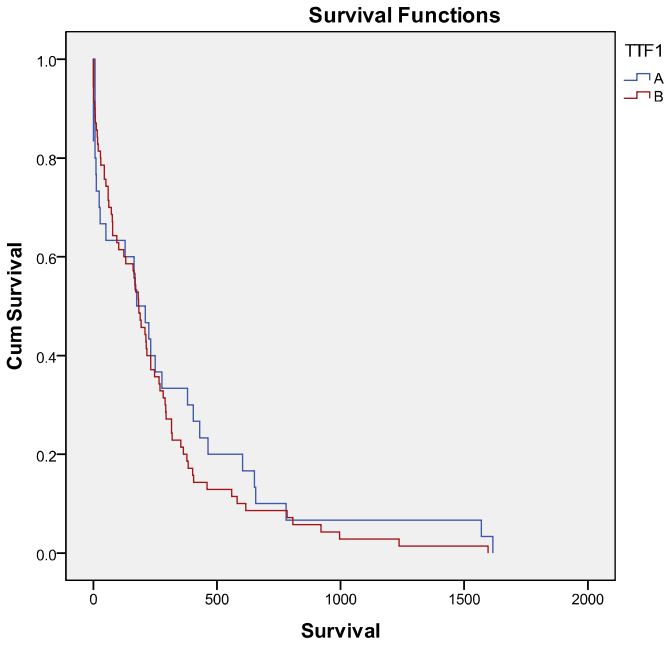

Fig. 13 – Survival Curves in TTF1.

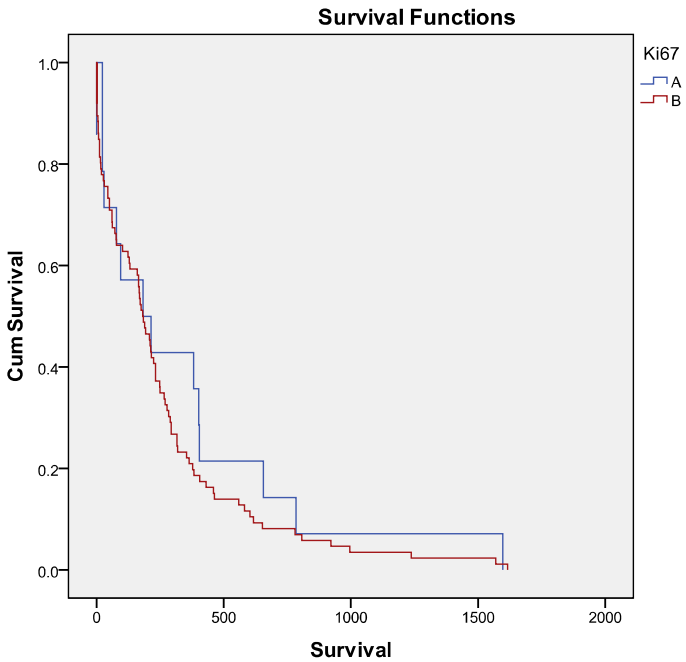

Fig. 14 – Survival Curves in Ki67

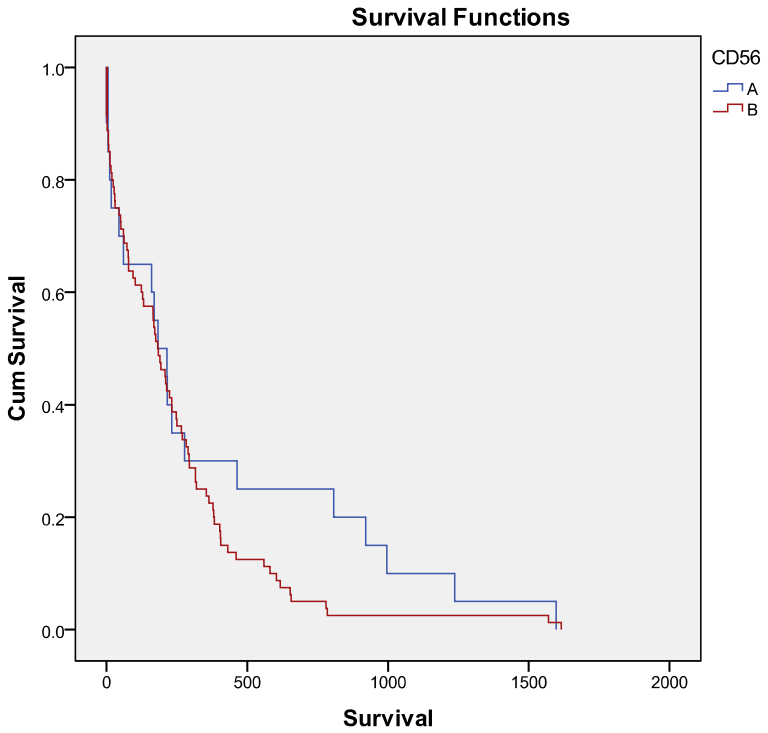

Fig. 15 – Survival Curves in CD56.

Combinations between two Imunohistochemical Markers were applied to avoid less then 5 cases per antibody and their significance is in Table IX. Each relation was established between a curve expressed by the group with the condition (C - combination between two Markers) and the curve formed by the rest of patients survival (D).

Overall Comparisons - Log Rank (Mantel-Cox)									
Marks Combination		Sig.	Survival Mean	nº	Marks Combination		Sig.	Survival Mean	nº
CromoA (A) +	CD56 (A)	0.087	D>C	12	CD56 (B) +	Ki67 (A)	0.943	D=C	11
	CD56 (B)	0.331	D>C	31		Ki67 (B)	0.258	D>C	69
	Ki67 (A)	0.543	C>D	5		TTF1 (A)	0.232	C>D	24
	Ki67 (B)	0.018	D>C	38		TTF1 (B)	0.026	D>C	56
	TTF1 (A)	0.520	D>C	16		CK7 (A)	0.254	D>C	45
	TTF1 (B)	0.100	D>C	27		CK7 (B)	0.940	D=C	35
	CK7 (A)	0.355	D>C	26		LP34 (A)	0.264	D>C	79
	CK7 (B)	0.126	D>C	17		LP34 (B)	-	-	1
	LP34 (A)	0.064	D>C	41		Total Patients			80
	LP34 (B)	-	-	2	Ki67 (A)	TTF1 (A)	0.728	C>D	5
	Total Patients			43		TTF1 (B)	0.492	C>D	9
CromoA (B) +	CD56 (A)	0,007	C>>D	8		CK7 (A)	0.996	C=D	11
	CD56 (B)	0.866	D>C	49		CK7 (B)	0.199	C>D	3
	Ki67 (A)	0.630	C>D	9		LP34 (A)	0.429	C>D	14
	Ki67 (B)	0.138	C>D	48		LP34 (B)	-	-	0
	TTF1 (A)	0,131	C>D	14		Total Patients			14
	TTF1 (B)	0.550	C>D	43	Ki67 (B)	TTF1 (A)	0.532	C>D	25
	CK7 (A)	0.981	C=D	31		TTF1 (B)	0.239	D>C	61
	CK7 (B)	0.05	C>D	26		CK7 (A)	0.448	D>C	46
	LP34 (A)	0.05	C>D	57		CK7 (B)	0.875	C=D	40
	LP34 (B)	-	-	0		LP34 (A)	0.487	D>C	84
	Total Patients			57		LP34 (B)	-	-	2
	Ki67 (A)	0.225	C>D	3		Total Patients			86
	Ki67 (B)	0.542	C>D	17	TTF1 (A)	CK7 (A)	0.492	D>C	19
	TTF1 (A)	0.381	D>C	6		CK7 (B)	0.089	C>D	11
	TTF1 (B)	0.077	C>D	14		LP34 (A)	0.358	C>D	28
CD56 (A) +	CK7 (A)	0.608	C>D	12		LP34 (B)	-	-	2
	CK7 (B)	0.283	C>D	8		Total Patients			30
	LP34 (A)	0.225	C>D	19	TTF1 (B)	CK7 (A)	0.808	D>C	38
	LP34 (B)	-	-	1		CK7 (B)	0.598	D>C	32
	Total Patients			20		LP34 (A)	0.492	D>C	69
CK7 (A)	LP34 (A)	0.450	D>C	57		LP34 (B)	-	-	1
	LP34 (B)	-	-	0		Total Patients			70
	Total Patients			57					
CK7 (B)	LP34 (A)	0.418	C>D	41					
	LP34 (B)	-	-	2					
	Total Patients			43					

Table IX - Test of equality of survival distributions for the different factors. C – condition (combination of two markers); D – the other patients that doesn't verify the condition C.
Nº - number of patients which verify the condition C in all 100 patients.;

Survival Mean						
Combination Marks	Sig.	Patients Group	Estimate	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
CromoA (A) + CD56 (A)	0.087	C	149.750	41.257	68.886	230.614
		D	290.761	37.706	216.857	364.665
CromoA (A) + Ki67 (B)	0.018	C	182.789	27.742	128.416	237.163
		D	329.645	50.720	230.233	429.057
CromoA (A) + LP34 (A)	0.064	C	201.195	32.009	138.458	263.932
		D	324.322	52.055	222.295	426.349
CromoA (B) + CD56 (A)	0,007	C	726.500	206.453	321.852	1131.148
		D	234.478	29.226	177.196	291.761
CromoA (B) + CK7 (B)	0.05	C	391.231	95.846	203.372	579.089
		D	232.595	30.007	173.780	291.409
CromoA (B) + LP34 (A)	0.05	C	327.667	53.829	222.161	433.172
		D	202.488	30.557	142.596	262.381
CD56 (A) + TTF1 (B)	0.077	C	473.286	141.072	196.784	749.787
		D	241.372	31.055	180.504	302.240
CD56 (B) + TTF1 (B)	0.026	C	200.804	24.155	153.460	248.147
		D	366.795	68.368	232.794	500.797
TTF1 (A) + CK7 (B)	0.089	C	469.455	175.029	126.399	812.511
		D	249.663	30.897	189.104	310.222

Table X – Combinations with significance and closed to significance meaning with their survivals and CI means. Blue – better prognosis; Orange – worse prognosis

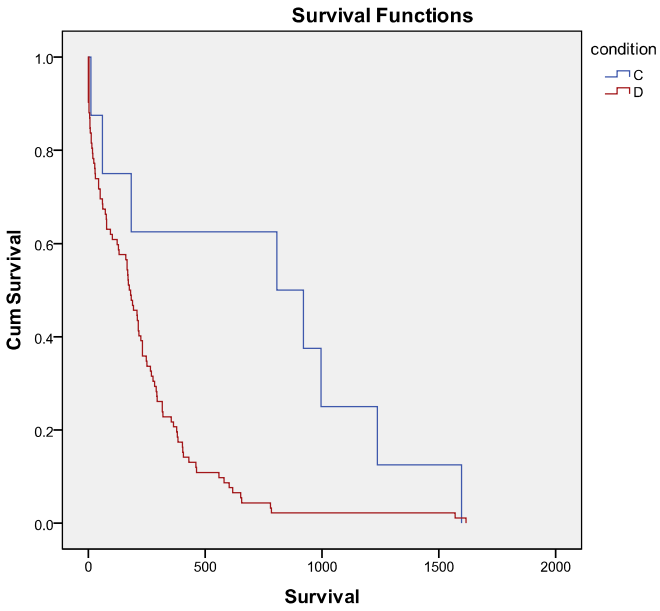

Fig.16 – Survival Curves of Population expressing CromoA B + CD56 A – (C) and rest of the patients (D).

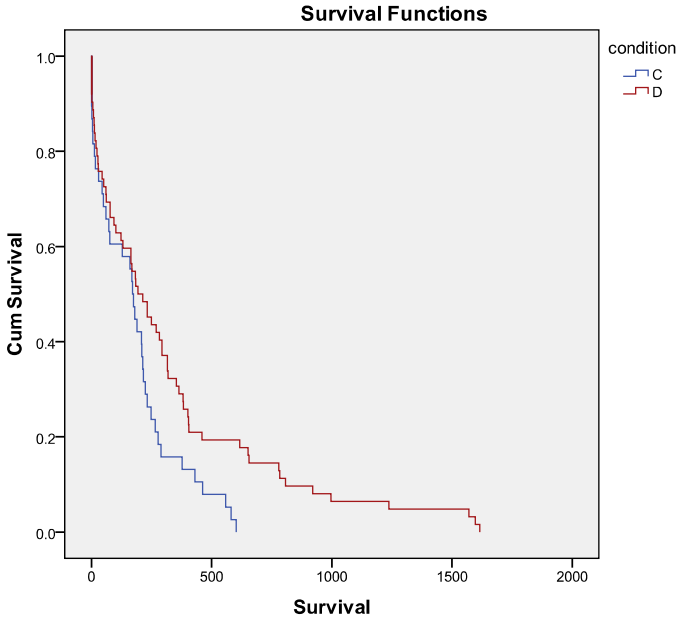

Fig.17 – Survival Curves of Population expressing CromoA A + Ki67 B – (C) and rest of the patients (D).

Discussion

Small Cell Lung Cancer remains an incurable disease for the majority of patients with a poor prognostic survival as demonstrated in this study as for the best of our knowledge is the first work trying to relate cell immunohistochemical characteristics and survival.

Extended disease was associated with significantly worse outcomes with a very significant value (0.000). The mean of survival with a LD-SCLC was 482 days (16months with a 95% Confidence Intervals from 10 months to 22 months) and survival mean in ED-SCLC was 182 days (6months with a 95% CI from 4 months to 8 months), slightly different from other reports. Nathan F. Sumithra J. et al (3) had a mean ranged from 17.2 months to 26.4 months for LD-SCLC trials and from 2.6 months to12.3 months for ED-SCLC trials. On the other hand extent of disease has traditionally been used for prognostic stratification of patients with SCLC. The current study demonstrated that staging which was named limited or extensive disease remains the most powerful prognostic factor for SCLC. (19)

Increased patients age and “being a man” was not associated with a better survival in this group of population and age curve had the most significant value for the two curves by 70 years. Female gender is another commonly described prognostic factor in SCLC (3). In the present analysis a different survival outcome related to gender could not be found.

In this trial SCLC was associated with male gender (87%), 65 years mean age and 81% of the patients were smokers with heavy smoking history (mean 61,3 pack-years). From the 13 women included only one was smoker and only five men were not smokers indicating the possible relation between smoking history and SCLC already described in other works.

As a large number of patients were diagnosed without symptoms the aggressive behavior of SCLC is explained as a silent disease till later stages.

A survival mean value of 274 days (9months) was determined, and median of 183 days (6months) for the whole population. According to Fig. 4 the upper and lower quartile were 37 days and 359 days, closed to mean value, but the range of survival values is very large. Most of the patients die in 12 months (76%) Fig 5. This work provides prognostic factors that at time of diagnosis could predict a better survival.

The six IMC Markers were applied to all 100 patients and divided in two variables: A – cellular expression till 25% of cells, and B – more than 25% of positive. The curves of A and B for each IMC Marker were compared individually in order to find difference influence in survival time of the patients that expressed B against A. Those curves were analyzed statistically and are exposed in Table VII. The patients showed a significant meaning in Chromo A curves isolated, with a mean of Survival with the group expressing A of 6,7 months and 11 months in the group expressing B. According to Drivsholm L. Paloheimo LI. and Østerlind K. (20): Survival in SCLC is significantly worse for patients with elevated Chromo A serological values and Chromo A is a significant prognostic factor – also in multivariable analysis. This study was not made with serological values of Chromo A but according to our results Imunohistochemical analysis of Chromo A in SCLC could be an indicator of good prognosis in SCLC.

Tumors that expressed Chromo A (B) & CD56 (A) had the best prognosis with a survival mean of 726 days (24,2months) against 234 days (7,8 months), better than the actual known survival of LD-SCLC. The worst prognosis was for the combination of CD56 (B) + Ki67 (B) with a survival mean of 6 months against 11 months for the rest of the

patients. Other combinations were also statistically significant. Cases expressing CD56 (B) & TTF1 (B) had worse prognosis with a survival mean of 6,7 months against 12 months; and the combination of Chromo A (B) & CK7 (B) had an improved in survival time with mean of 13 months against 7,7 months for the rest of the patients.

It was clearly demonstrated that neuroendocrine differentiation based in Chromo A expression by tumor cells indicates an higher survival as showed in this statistical study. The cases expressing Chromo A in more than 25% of cells have improved survival when compared to B group (less than 25% of positive cells). The best survival curve was seen in Chromo A (B) & CD56 (A). Our results are consistent with previous studies that showed CD56 role in cell surface adhesion, making its expression related to bad prognosis. (17) The combination of "no expression" of CD56 – A - and highly expressed ChromoA – B - makes the best survival time. Combination between Chromo A (B) and CK7 (A) also had a better survival; fact that could be explained by the relation of CK7 and pulmonary adenocarcinomas improving the prognosis of this tumor. It is well reported the relation between CK7 and Adenocarcinomas and is also known the best prognosis of this tumor when compared with SCLC.

Ki67 had a role in proliferation rate determination as explained in many previous works and related with worse outcomes in survival times of the patients expressing more then 25% of positive cells. This fact could be the reason for CD56 (B) + Ki67 (B) worst prognosis. (18)

The high expression of TTF1 tended to show better survival then TTF1 (-) group in non SCLC (21). That was not verified in this trial.

The utility of these 6 antibodies applied in routine diagnosis of SCLC, either by cytology in biopsies reinforces de utility of IBPCC (Immunohistochemical Bronchial Pulmonary Carcinoma Classification). It is a simple and efficient tool for streamlining the registration of lung cancer histological characteristics in biopsies and other reduced samples to support clinical evidence and trials. (22)

References

1. Ries LAG. Harkins D. Krapcho M. Mariotto A. Miller BA. Feuer EJ. et al. SEER Cancer Statistics Review, 1975-2004. Lung Bronchus, Percent Distribution and Counts by Histology, 2001-2004, Both Sexes by Race, Table XV-23.
2. Kakolyris S. Agelidou A. Androulakis N. Tsaroucha E. et al. Cisplatin plus etoposide chemotherapy followed by thoracic irradiation and paclitaxel plus cisplatin consolidation therapy for patients with limited stage small cell lung carcinoma 2006; Lung Cancer (2006) 53, 59-65.
3. Nathan R. Sumithra J. Steven E. et al. Prognostic factors Differ by Tumor Stage for Small Cell Lung Cancer. American Cancer Society 2009; 2721-2731
4. Johnson BE. Bridges JD. Sobczeck M. et al. Patients with limited stage small-cell lung cancer treated with concurrent twice-daily chest radiotherapy and etoposide/cisplatin followed by cyclophosphamide, doxorubicin, and vincristine. 1996; 14:806-813.
5. Murray N. Coy P. Pater JL. et al. Importance of timing for thoracic irradiation in the combined modality treatment of limited-stage small cell lung cancer. The National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol. 1993; 11:336-344.
6. Turrisi AT. Kim K. Blum R. et al. Twice-daily compared with once-daily thoracic radiotherapy in limited small.cell lung cancer treated currently with cisplatin and etoposide. N. Engl J Med. 1999;340:265-271

7. Lassen U. Osterlind K. Hansen M. et al. Long term survival in small cell lung cancer: post treatment characteristics in patients surviving 5 to >18 years – an analysis of 1714 consecutive patients. J Clin Oncol. 1995; 13; 1215-1220.
8. Albain KS. Crowley JJ. LeBlanc M. Livingston RB. Determinants of improved outcome in small cell lung cancer: an analysis of the 2580-patient Southwest Oncology Group data base. J Clin Oncol. 1990;8 1563-1574.
9. Cerny T. Blair V. Anderson H. Bramwell V. Thatcher N. Pretreatment prognostic factors and scoring system in 407 small cell lung cancer patients . Int J Cancer, 1987;39:146-149
10. Rawson NS. Peto J. An overview of prognostic factors in small cell lung cancer. A report from de Subcommittee for the Management of Lung Cancer of the United Kingdom Coordinating Committee on Cancer Res. Br J Cancer. 1990; 61:597-604.
11. Sculier JP. Chansky K. Crowley JJ. Van Meerbeeck J. Goldstraw P. The impact of additional prognostic factors on survival and their relationship with the anatomical extent of disease expressed by the 6th edition of TNM Classification of malignant Tumors and the proposals for the 7th edition. J Thorac Oncol. 2008; 3:457-466.
12. Souhami RL. Bradbury I. Geddes DM. Spiro SG. Harper PG. Tobias JS. Prognostic significance of laboratory parameters measured at diagnosis in small cell carcinoma of the lung. Cancer Res. 1985;45:2878-2882.

13. Wolf M. Holle R. Hans K. Drings P. Havemann K. Analysis of prognostic factors in 766 patients with small cell lung cancer: the role of sex as a predictor for survival. Br J Cancer, 1991;63:986-992.
14. Gregorc V. Spreafico A. Floriani I. Colombo B. e tal – Prognostic value of circulating Chromogranin A and soluble tumor necrosis factor receptors in Advanced nonsmall cell lung cancer. American Cancer Society 2007 845-853.
15. Tarek G. Guoan C. Hong W. Proteomic Analysis of Cytokeratin Isoforms Uncovers Association with Survival in Lung Adenocarcinoma. Neoplasia 2002; 4 (5): 440-448
16. Loreto C. Lauro V. Puglisi F. Damante G. Fabbro D and Beltrami C. – Immunocytochemical expression of tissue specific transcription factor-1 in lung carcinoma. J. Clin Pathol 1997; 50:30-32
17. Kontogianni K. Nicholson A. Butcher D. Sheppard M. – CD56: a useful tool for the diagnosis of small cell lung carcinomas on biopsies with extensive crush artefact. J Clin Pathol 2005; 58:978-980.
18. Nguyen X. Lee W. Chung J. Park S. Sung S. Kim Y. So Y. et al – FDG uptake, glucose transporter type 1, and ki67 expression in non-small-cell lung cancer: correlations and prognostic values. European Journal of Radiology 2007; 62: 214-219.
19. Arinc S. Gonlugur U. Devran O. Erdal N. et al – Prognostic factors in patients with small cell lung carcinoma. Med Oncol 2009.

20. Drivsholm L. Paloheimo L. Østerlind K. - Chromogranin A, a significant prognostic factor in small cell lung cancer. British Journal of Cancer (1999); 81(4), 667–671.
21. Myong NH. – Thyroid Transcription Factor-1 (TTF1) expression in human lung carcinomas: its prognostic implication and relationship with expressions of p53 and Ki67 proteins. J Korean Med Sci 2003; 18:294-500.
22. Carvalho L. Reclassifying bronchial – pulmonary carcinoma: differentiating histological type in biopsies by immunohistochemistry. Rev Port. Pneumol 2009; 15 (6):1101-1119.

Agradecimentos

Os meus agradecimentos à Sra. Professora Doutora Lina Carvalho e à Sra. Dra. Alice Mariano Pêgo por toda a atenção e disponibilidade demonstrada no decorrer da elaboração do presente trabalho.